REMARKS

Reconsideration is respectfully requested in view of the foregoing amendments and the remarks which follow.

Claims 30-32, 35-38, 46-53 and 62 have been amended herein. Claims 63-72 have been added. Claims 33, 48 and 49 have been cancelled. Claims 34, 39-45 and 54-61 have been withdrawn.

The claims presently pending in the application are 30-32, 35-38, 46, 47, 50-53 and 62-72.

The objections to claims 30, 31, 48 and 53 have been overcome by cancelling reference to the non-elected SEQ. ID NOS: 2, 4, 6 and 8. Accordingly, the objection should be withdrawn.

The objection to claims 30, 31, 33, 35, 38, 48, 52 and 53 for using an improper Markush expression has been overcome by employing the proper terminology. Accordingly, this objection should also be withdrawn.

Independent claim 30 has been amended to refer specifically to a monoclonal antibody (mAb) for the indicated uses, the claimed mAb recognizing and *inhibiting* the activity of BAG3 protein, and also recognizing at least one BAG3 fragment of amino acid sequence consisting of SEQ ID NO:15, 16, 17 or 18. (This replacement of the feature of antibody 'modulation' of BAG3 activity by antibody 'inhibition' of BAG3 activity, and the corresponding increase in apoptosis as demonstrated in the Examples of the present application, is consistent throughout the amended claims: *e.g.* claim 32.)

Claim 31, as amended, is a dependent claim in which the subject matter is now encompassed by the scope of amended claim 30 (*i.e.* antibodies which bind peptide sequences SEQ ID NO:15-18, but which also bind to the specified partially homologous sequences).

Claim 33 has been cancelled and aspects of its subject matter, namely in which the antibody of claim 30 is for treating diseases characterized by increased BAG3 activity or decreased apoptosis, are now the subject of new claims 69-72.

Claims 46-52 are directed to compositions which comprise a pharmaceutical carrier. Claims 48-49 have been cancelled as their subject matter is rendered redundant by the technical features of amended claims 30 and 46. In claims 50-52 the uses to which the composition is applied have been amended to comply with the BAG3 inhibition activity of the antibody described above.

Independent claim 53 and new, dependent claims 63 to 68 are directed to a diagnostic agent of the invention defined in accordance with amendments to the

antecedent claims. In the dependent claims the uses to which the diagnostic agent is applied are amended to comply with the BAG3 inhibition activity of the antibody described above.

New claims 69-72 are directed to the antibody of claim 30 for use in treating diseases which are defined to comply with the amendment to claim 30 (antibody effect on BAG3 activity restricted to inhibition) and are consistent with those uses to which the claimed diagnostic agent and claimed compositions are applied.

Addressing Issues Raised in the Detailed Action

1. Election / Restriction

The requirement of restriction to Invention I, as drawn to antibodies that recognize BAG3 protein and fragments thereof selected from the following SEQ ID NOs:15, 16, 17, 18 is complied with throughout the set of newly amended claims filed herewith.

2. Status of the Claims

The subject matter of the set of newly amended claims filed herewith is directed to that of the previous independent claims shown below, in correspondence to dependent claims of the newly amended set:

independent claim 30: monoclonal antibody; dependent claims 31,32,35,36,69,70,71,72;

independent claim 37: hybridoma mother clone; – ;

independent claim 38: peptide construct; – ;

independent claim 46: composition; dependent claims 47, 50, 51, 52;

independent claim 53: diagnostic agent; dependent claims 63, 64, 65, 66, 67, 68;

independent claim 62: kit; – ;

3, 4. Claim Rejections 35 U.S.C. §112, first paragraph: (Written Description)

Examined claims 31 and 62 are rejected under 35 U.S.C. §112, first paragraph as failing to comply with the written description requirement. This rejection is respectfully traversed.

(a) Claim 31 has been amended so that the subject matter of claim 30 is appropriately dependent upon that of independent claim 30. *i.e.*, the antibody

of claim 31 as newly amended, possesses all the properties of the antibody of claim 30 (such as, for example, the recognition of at least one BAG3 protein fragment with amino acid sequence consisting of one of the sequences SEQ ID NO:15, 16, 17 or 18) but additionally is able to recognize a peptide "in which the amino acid sequence of said peptide consists of a sequence having a homology of at least 75% [...] to at least one sequence selected from the group of sequences consisting of SEQ ID NO:15, 16, 17 and 18."

Thus the antibodies of newly amended claim 31 are clearly antibodies of the present, elected invention, but which, while recognizing and binding to the peptides of at least one of SEQ ID NO:15, 16, 17 and 18, may do so with a selected (*e.g.* reduced) affinity in comparison to antibodies which bind exclusively to a peptide with a sequence selected from SEQ ID NO:15-18 (for example an antibody recognizing a particular secondary or tertiary structure formed by the peptide to which it binds).

(b) Amendment of claim 62 has removed the phrase "... and functionally equivalents of the above identified sequences", thereby removing the cause of this rejection.

For the foregoing reasons, the rejections of claims 31 and 62 under 35 U.S.C. §112, first paragraph, with respect to written description have been overcome and should be withdrawn.

5. Claim Rejections 35 U.S.C. §112, first paragraph: (Enablement)

Examined claims 30-33, 35-38, 46-53, and 62 are rejected under 35 U.S.C. §112 first paragraph as failing to comply with the enablement requirement. The Examiner maintained that the use of the antibody of the invention for therapeutic purposes would require "undue experimentation." This rejection is traversed.

The antibodies of the invention as claimed in the newly amended set of claims are monoclonal antibodies which recognize BAG3 protein and which modulate the BAG3 protein by (specifically) inhibiting it. The antibodies of the invention are selected by their ability to recognize peptide sequences that are unique to the BAG3 protein. That these sequences are unique is indicative of a role supporting the structural and/or functional characteristics of BAG3 protein. Consequently, the binding of a large antibody molecule to an epitope located within such a sequence as part of the BAG3 protein is most likely to have a disruptive effect upon both the structure and function of BAG3.

Claim 30 has therefore been amended to replace the term 'modulate' with that of 'inhibit' with regard to the effect of the claimed antibody upon the activity of the BAG3 protein. The person skilled in the art and aware of the disclosure of the present application would not require undue experimentation to obtain such an antibody.

The types of disease for which the antibody of the invention (newly amended claims 32, 69-72) – or the composition comprising it (newly amended claims 50-52) – may be used has been amended to relate to those types of disease, and specific examples thereof, in which BAG3 inhibition would generate the desired result, namely, an increase in apoptosis, a diminishment of proliferative symptoms, and recession of the disease itself: *i.e.* neoplastic disease (*e.g.* leukemia, osteosarcoma) and autoimmune disease.

Results of antibody detection of variation from normal values of BAG3 protein in cells over-expressing BAG3, in leukemic cells, and in leukemic cells subject to BAG3 down-regulation by specific anti-sense oligodeoxynucleotides are all provided in the original as-filed specification (see, for example, page 12 line 1 to page 13 line 6 and page 18 line 3 to page 19 line 10.)

The attached publication by Lopez-Guillermo & Mercadal (European Society for Medical Oncology, 2007) shows that in the past few years, antibody therapy has become well established, particularly for treating haematological disease. At Table 2, Lopez-Guillermo & Mercadal show that it is quite normal for an antibody dosage to be varied depending upon the response of the particular patient. While some of the five exemplified antibodies are recommended for varying lengths of treatment, radiolabelled tositumomab (Bexxar(R)) is shown to require dosimetry to establish the necessary dose Hence, the person skilled in the art is familiar with techniques of determining and varying the amount of antibody to prescribe depending upon its targeting effectiveness in the individual patient, and/or the response to the radiolabel it may carry, and/or any side effects that an individual patient may display.

In short, the person (physician) skilled in this art is familiar with the need to determine an appropriate dosage when initiating and maintaining antibody therapy, and would regard this as a normal procedure, and not as one incurring undue experimentation.

We note that the Lopez-Guillermo document was published in 2007. However, it is a review of antibody therapeutics which are currently approved for use, which have therefore completed a number of years of clinical trials. At the date that the present invention was made, the person skilled in the art would have been able to determine an appropriate therapeutic regime for treatment with a given antibody without undue experimentation, but would have expected some initial estimation and iteration to be required, depending upon the response of individual patients.

Indeed, Rituximab was initially approved by the FDA in 1997 (see: http://en.wikipedia.org/wiki/Rituximab) and comprises a humanised monoclonal antibody.

Applicants also note that the reshaping of human IgGI antibody for serotherapy in humans by introducing hypervariable regions from rat antibody

directed against human lymphocytes was disclosed back in 1988 by Riechmann et al. (1988) 332 (612): 323-327, indicating that therapeutic antibody technology has a substantial history of development.

The increase of apoptosis in leukemic cells by BAG3 antisense oligonucleotides reducing expression of BAG3, as confirmed by detection of BAG3 levels using immunofluorescent antibody, indicates that other means of inhibiting BAG3 activity, for example by antibody binding, would provide alternative means for increasing apoptosis in leukemic cells, or in reducing osteosarcoma tumor size (see, for example, page 12 line 5 to page 15 line 8 regarding modulation of BAG3 expression (and hence activity) in primary leukemia cells and in osteosacoma cells transplanted into mice).

The diagnostic agents of the present invention use antibody of the invention for binding to and detecting BAG3 protein. The diagnostic agent claims are therefore directed to diagnosis of diseases in which the normal level of BAG3 changes detectably, whether due to an increase or a decrease. The diagnostic agent may therefore be used to detect BAG3 variation in diseases associated with either an increase or a decrease in apoptosis.

For the foregoing reasons, the rejection of claims 30-33, 35-38, 46-53, and 62 under 35 U.S.C. §112, first paragraph, with respect to enablement has been overcome and its withdrawal is solicited.

6. Claim Rejections 35 U.S.C. §112, second paragraph: (Indefiniteness)

Claims 30-33, 35-38, 46-53, and 62 stand rejected under 35 U.S.C. §112 second paragraph as being indefinite. This rejection is respectfully traversed.

(a) Claim 30 as amended no longer refers to "Isolated antibodies that ... modulate BAG3 protein", but rather refers specifically to "Monoclonal antibody ... wherein said antibody recognizes BAG3 protein and inhibits BAG3 protein activity". The recognition of the BAG3 protein by the antibody is further defined in amended claim 30.

Hence, the lack of clarity relating to the word "modulate" is rendered moot.

(b) The wording of claim 38 has been amended to render definite the subject matter, namely a peptide construct which is a Multiple Antigen Peptide (MAP) selected from a defined group of such MAPs. The rejection of claims dependent upon or referring to the subject matter of claim 30 is, as a consequence, similarly clarified and rendered definite.

(c) Claim 53 has been amended to indicate what the diagnostic agent will diagnose, namely, "a disease characterized by modulation of BAG3 protein expression", this definition being further refined in dependent claims 63-68.

(d) Claim 62 has been amended by, *inter alia*, removing the terminal phrase "... or functionally equivalents of the above identified sequences." which led to the characterization that the claim was indefinite.

For the foregoing reasons, the rejection of claims 30-33, 35-38, 46-53, and 62 under 35 U.S.C. §112, second paragraph, has been overcome and its withdrawal is respectfully solicited..

7. Claim Rejections 35 U.S.C. §102(b)(e): (Novelty)

Claims 30-33, 46, 49-52 and 62 are rejected under 35 U.S.C. 102(e) as being anticipated by Reed & Takayama (U.S. Patent 6,696,558) and under 35 U.S.C. 102(b) as being anticipated by Kohn *et al.* (U.S. Patent 5,652,223). These rejections are respectfully traversed.

Although both the disclosure of Reed & Takayama ("the '558 patent"), and that of Kohn *et al.* ("the '223 patent") disclose methods by which a skilled person might proceed to obtain antibodies to a protein having a sequence equal to (the '558 patent) or very similar to (the '223 patent) that of the BAG3 protein, neither document discloses the isolation of a particular antibody, or any characteristics of such an antibody. These disclosures are therefore hypothetical.

The disclosure of the '558 patent also refers to the generation of polyclonal and monoclonal antibodies from peptide fragments of the BAG3 protein.

However, the claims of the present application, as newly amended, are directed to a particular, limited selection of narrow range from these generalized disclosures, which refer to the entire population of antibodies that might be generated against the mass of possible epitopes collected within the full-length polypeptide chain of the BAG3 protein, and/or within any number of undefined fragments thereof.

The disclosure of the present application indicates that peptides used to generate the antibodies of the present invention, as amended and filed herewith, were those having an amino acid sequence consisting of one of SEQ ID NO:15, 16, 17 or 18. The antibodies claimed are particularly monoclonal antibodies which are characterized, *inter alia,* by their ability to recognize a BAG3 fragment of amino acid sequence consisting of the sequence of one of SEQ ID NO:15, 16, 17 or 18.

Neither of the cited prior art documents discloses such a monoclonal antibody.

Furthermore, the particular selection of the indicated narrow range of antibody that the amended claims select from the sum of possible antibodies that might be generated against the totality of epitopes contained within the BAG3 protein sequence is a purposive selection.

The particular selection of amino acid sequences used for the target peptides which were employed to generate the antibodies of the invention is itself characterized by selective specificity for BAG3, and appropriate size for use in generating MAP constructs for immunogen preparation. The selection criteria used, namely the ability to bind to one of the indicated four short BAG3 peptides, select for a group of antibodies which possess the advantageous characteristics indicated below.

These characteristics indicate that the antibodies of the invention are, in themselves, a purposive selection of a narrow range chosen from the entirety of anti-BAG antibodies that might have been known or determined by those of ordinary skill in the art.

Moreover, as discussed above, these antibodies of the present invention as newly claimed, are monoclonal antibodies, namely, antibodies with the indicated combination of advantageous characteristics that have not been disclosed in the prior art, nor could they reasonably be expected to be selected by any arbitrary means.

Thus, the purposive selection of the antibodies of the present invention as newly claimed, with their combination of technical characteristics, selects for antibodies with the following advantageous properties:

(a) Selective specificity for BAG3:

Each of the peptide sequences to which the antibodies bind is unique to BAG3 (see page 18, lines 15-16).

Consequently:

(i) antibodies binding to these selected sequences will exhibit low cross-reactivity with related proteins, reducing interference when using the antibodies of the invention in sensitive diagnostic assays and minimizing artifacts and side-effects when using them as research reagents and therapeutic agents, respectively; and

(ii) antibodies binding to these selected sequences are more likely to interact with a structural feature unique to BAG3 protein, and in so doing, to affect a function unique to BAG3 protein. The antibodies of the invention are, therefore, purposively selected to perform the biochemical functions for which they are intended, and as specified in the amended claims, such as inhibiting BAG3 protein activity.

The carboxy-terminal region of BAG-type proteins incorporates a 'BAG' domain common to BAG-type proteins. Nevertheless, the carboxy-terminal peptide sequences used for generating the antibodies of the invention (and to characterize them in that they bind to peptides consisting of these sequences) lie outside of the consensus sequences of the BAG domain and outside of other

domains held in common by the BAG family of proteins (see, for example, Reed & Takayama at Fig. 10A). The sequences relating to the presently claimed invention are selected to be unique to BAG3, and are located outside of the BAG domain (page 18, lines 15-16).

(b) Suitability for generation from MAP construct immunogens:

(i) Each of the sequences used to define the binding or recognition characteristics of the antibodies of the newly amended claims, is of limited length: namely 15 to 16 amino acid residues (page 18 lines 3-12).

(ii) This provides the following functionality: the peptide recognized by the antibody may be conveniently used as an immunogen for generating the antibody; and, in the case of monoclonal antibodies, as a basis for ultimately generating the hybridoma which produces the monoclonal antibody. As each of the sequences used to define the recognition characteristics of the claimed monoclonal antibodies is of limited length, namely, 15 to 16 amino acid residues (see (i), above), peptides that may be used to generate the monoclonal antibodies of the invention, which have the identical sequence, will be of an appropriate length to be used for constructing Multiple Antigenic Peptide (MAP) constructs.

Immunizing mice with MAP constructs results in significant enhancement of the antigenic peptides and enables the generation of particularly efficient (*i.e.* high affinity) antibodies, which itself is important for detecting proteins expressed in low amounts (present application, page 18, lines 17-22). Under physiological or pathological conditions in primary cells, many important proteins are expressed only at low levels, particularly proteins having regulatory functions.

(c) High specificity antibody binding provides mapping capability for BAG3 interactions:

Monoclonal antibodies provide the necessary specificity of binding to be able to map the protein epitopes involved in the interaction of BAG3 with its multiple molecular binding partners (present application; page 19, lines 4-8).

(d) Selective functional effect of antibody binding upon BAG3 activity:

Monoclonal antibodies can effect specific agonistic or antagonistic (of relevance to the antibodies of the newly amended claims) influences upon the biological functions of a protein (for example, by steric inhibition) which may modulate BAG3 activity in mechanisms determining cell survival and/or death (present application; page 19, lines 7-10).

The foregoing explanatory commentary demonstrates that the purposive selection represented by the antibodies of the present invention, as newly claimed, provides these antibodies, as well as the claims directed to them and the claims

referring to them, with the properties of being both novel and non-obvious over Reed & Takayama (the '558 patent) and also with the properties of being both novel and non-obvious over Kohn *et al.* (the '223 patent).

Furthermore, we note that the disclosure of Kohn *et al.* (the '223 patent), in fact teaches away from the present invention. The '223 patent teaches that BAG3 protein inhibits the assistance provided by hsp70 chaperone activity for the refolding of test proteins, which would suggest that BAG3 protein should inhibit refolding and therefore inhibit cell survival ('223 patent at column 17, line 58 to column 19, line 9). In contrast, the present application demonstrates that ***BAG3 protein activity inhibits apoptosis, resulting in increased cell survival.*** Direct evidence for these phenomena is provided by proliferative cells, namely osteosarcoma cells cultured *in vivo* in a mouse model, and in primary leukemia cells.

Each of the objections and rejections in the August 6, 2007 Office Action have been addressed and either overcome or rendered moot by the combination of the claim amendments and the accompanying arguments.

Therefore, for all of the foregoing reasons, claims 30-33, 35-38, 46-53, and 62 distinguish over the teachings of Reed & Takayama and Kohn et al. Accordingly, the rejections under 35 U.S.C. § 102(b) and § 102(e) have been overcome and should be withdrawn since the Examiner has not established a *prima facie* case of anticipation.

The issuance of a Notice of Allowance is respectfully solicited.

Please charge any fees which may be due and which have not been submitted herewith to our Deposit Account No. 01-0035.

Respectfully submitted,

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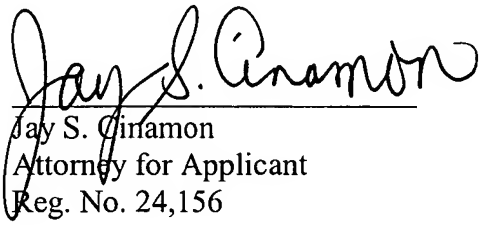
By 
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The clinical use of antibodies in haematological malignancies

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introduction

Chemotherapy and radiotherapy have, for decades, been the conventional treatment for patients with haematological malignancies, including lymphomas [1] and leukaemias [2]. Stem-cell transplantation (particularly allogeneic) also opened new therapeutic possibilities in selected cases. More recently, the treatment of these patients with monoclonal antibodies (MoAbs) has provided an effective alternative approach, which can be combined with the above mentioned therapies [3–5]. MoAbs are the first targeted treatment against the cancer that is effective and gives the possibility of reducing non-specific toxicity. In recent years a number of new MoAbs against different targets in haematological and non-haematological malignancies, including lymphoid and myeloid tumours, have been progressively incorporated to the therapeutic armamentarium [6–11].

A review of the present status of MoAb therapy in all the haematological malignancies is very complicated, since the number of different MoAbs and, therefore, the number of clinical trials with these alone, and in combination, is exponentially increasing. Therefore, the objective of this manuscript is to review the state-of-the-art MoAb treatment, mainly focusing on those antibodies that are currently in use in clinical practice and, particularly, those already currently essential to the treatment of patients with leukaemias or lymphomas.

targets and antibody effector mechanisms

Different antigens can be the target of MoAbs in leukaemias and lymphomas. In Table 1, some of the antigens potentially interesting in clinical practice are listed. The list is not complete because the number of possibilities is large. The optimal target for MoAb therapy would be a specific antigen present at high density on tumour cells, absent or present at low concentrations on normal cells (or present in non-critical host cells), with stable expression and with no modulation or internalization. CD20 antigen, present in most B-cell neoplasias, has all of these requisites and is the paradigm of the target molecules [6, 12, 13]. Nevertheless, even in those cases with a lack of high tumour specificity, MoAbs offer the possibility of lower toxicity, compared with conventional chemotherapies, by increasing the therapeutic index and with no overlapping toxicity with the standard drugs [6].

Native unconjugated antibodies were the first ones used in clinical practice. The mechanisms of action to kill the tumour cells include complement-dependent cytotoxicity (CDC) (affected by antibody isotype and species, and antigen density), antibody-dependent cellular cytotoxicity (ADCC) [which requires specific isotypes and adequate ratios of effector cells, including natural killer (NK) cells, macrophages and polymorphonuclear cells, to target cells] and receptor-based signalling [12–17]. In addition, certain characteristics of the antibodies may modulate their activity. Thus, a humanized (at least chimeric) MoAb will have less immune response from the host by not producing inactivating antibodies. More recently, in order to increase the anti-tumour effect, MoAbs have been conjugated either with radiation emitters or with cytotoxins [18–21]. The latter could substantially increase the cytotoxic capability, but also the toxicity for the patient. Radio-immunotherapy with beta-emitting isotopes, such ^{131}I or ^{90}Y, has the advantage of making a 'crossfire effect' eliminating tumour cells to which the MoAb is not directly bound, although of course, the dose-limiting toxicity to normal cells is important [10, 21]. Cytotoxins conjugated to MoAb, such as calicheamicin (anti-tumour antibiotic), or toxins such as ribosomal inhibitory proteins (e.g. ricin, gelonin) are drugs that require entry to the cell to work, but once inside are extremely toxic for tumour cells.

Another aspect to point out regarding the treatment with MoAb is the possibility of additive or synergistic effect with some of the standard chemotherapies [22, 23]. This is important since the synergistic effect could be obtained with no substantial increase in toxicity. The advances during the last years in immunotherapy of haematological malignancies derive from the combination of MoAbs with conventional chemotherapy.

lymphoproliferative disorders

rituximab

Rituximab is a chimeric monoclonal immunoglobulin G1 antibody of humanized murine origin and targets the cell surface receptor CD20. It was the first antibody widely used in patients with malignant and non-malignant diseases. In the lymphoma setting, since its first use in humans a decade ago, rituximab has become an essential component of the therapy in all types of B-cell lymphomas, probably representing the major advance in this field since the use of doxorubicin in the 1970s [1, 3, 4]. Alone or in combination with

Table 1. Antigens that could be used as targets in haematological malignancies

Antigen	Distribution on normal cells	Haematological malignancies
CD2	T-cells, NK-cells	T-cell NHL, ALL
CD4	Subpopulation T-cells	T-cell NHL
CD5	T-cell and subpopulation of B-cells	CLL, T-cell NHL
CD19	B-cells	B-cell NHL
CD20	Mature B-cells	B-cell NHL
CD22	Precursors and mature B-cell; basophiles	B-cell NHL
CD23	B-cell subpopulation	CLL
CD40	Mature B-cells	B-cell NHL
HLA-DR	B-cells and monocytes	B-cell NHL, ALL, AML
CD52	Panleucocytic antigen	B- and T-cell NHL
CD80	Activated B- and T-cells, dendritic cells, APCs	B- and T-cell NHL
CD25 (IL-2 R)	Activated B- and T-cells, activated monocytes	B-and T-cell NHL (cutaneous lymphomas)
CD30	Activated B- and T-cell; monocytes	B- and T-cell NHL ($CD30^+$)
CD45	Most lymphoid and myeloid cells of various stages of maturation	AML, ALL, B- and T-cell NHL
CD33	Myeloid progenitors, monocytes, dendritic cells	AML
CD66	Normal and activated myeloid cells	Not AML, ALL
CD15	Myeloid and monocytic cells, epithelial cells	AML
CD13	Broad myeloid and monocytic expression, epithelial and stromal cells	AML
VEGF	Angiogenic molecules	B- and T-cell NHL, ALL, AML

NK, natural killer; NHL, non-Hodgkin's lymphoma; ALL, acute lymphoblastic leukaemia; CLL, chronic lymphocytic leukaemia; AML, acute myeloid leukaemia; APCs, antigen-presenting cells; VEGF, vascular endothelial growth factor.

chemotherapy, rituximab has dramatically improved the prognosis of patients with indolent and aggressive lymphomas.

The murine portion of the antibody, the Fab variable region, binds CD20 on the surface of the lymphoma cells with a high degree of specificity. The human portion of the antibody, the Fc region of the IgG1 immunoglobulin isotype, is then responsible for stimulating the immune pathways of the patient. The mechanisms of action, although not completely understood, include CDC, ADCC and receptor-based signalling, and, as already mentioned, rituximab shows synergy with other drugs [12, 17, 22, 23]. The appearance of inactivating antibodies against rituximab is rare and does not represent a problem in clinical practice.

The regimen of administration of rituximab is indicated in Table 2.

The main side effect is the infusion syndrome, seen during or in the next few hours after infusion, particularly during the first administration. It includes fever, chills, dizziness, nausea, itching, swelling of the throat, cough, fatigue, hypotension or transient bronchospasm [24–26]. The most severe form of presentation is the 'cytokine release syndrome', observed in patients with circulating malignant cells [27]. The severity of the syndrome directly depends on the number of circulating cells. Nevertheless, this complication is the exception and, in general, the infusion syndrome, if present, is mild and does not appear in the following infusions.

Another common toxicity is the depletion of normal CD20-positive lymphocytes from the blood, bone marrow and lymph nodes. However, at least for standard doses, this depletion does not compromise immunity: immunoglobulin levels do not significantly vary and there is no increased risk for infections, with the exception of some viruses like herpes virus, cytomegalovirus and hepatitis B virus. The toxicity in long-term treatment with rituximab (for instance, in the maintenance setting) is still under investigation.

CD20 is present in most B-cell lymphoproliferative disorders. Rituximab was first designed for indolent lymphomas {mainly follicular lymphoma (FL) [28–38]}, but its use has been progressively expanded to all B-cell CD20-positive malignancies, including diffuse large B-cell lymphomas (DLBCL) [39–44], mantle-cell lymphomas [35, 45, 46], MALT lymphomas [47], Waldenström disease, chronic lymphocytic leukaemia (CLL) [48–54], some types of Hodgkin's lymphomas, and also to some T-cell tumours such angioimmunoblastic lymphoma.

follicular lymphoma (FL). Rituximab was first assayed as monotherapy in FL. In the pivotal study performed in previously treated patients with low-grade lymphomas (mostly FL) [28], a response rate of 48% was observed, including 6% of complete response (CR). The response duration lasted for a median of 12 months. Patients relapsing after rituximab were re-treated with the same drug with similar responses and a few side effects. For untreated low-risk FL, the response rate with rituximab was 73%, including 26% of CRs and a proportion of molecular responses [31]. About 20% of responders maintained the response 7 years after rituximab [55]. Thus, single agent rituximab is at least as good as the majority of chemotherapies in this setting.

The combination of rituximab plus standard chemotherapy dramatically increases the CR rate, failure-free and disease-free survival both in previously treated and untreated patients. The latter was observed with different regimens {CVP (cyclophosphamide, vincristine and prednisone) [33], CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) [34], FCM (fludarabine, cyclophosphamide and mitoxantrone)

Table 2. Pharmacological characteristics of the most widely used monoclonal antibodies in haematological malignancies

	Rituximab (MabThera®)	[^{90}Y]Ibritumomab tiuxetan (Zevalin®)	[^{131}I]tositumomab (Bexxar®)	Alemtuzumab (MacCampath®)	Gemtuzumab ozogamicin (Mylotarg®)
Target antigen	CD20	CD20	CD20	CD52	CD33
Type of antibody	Chimeric	Radiolabeled murine	Radiolabeled murine	Chimeric	Recombinant humanized conjugated to calicheamicin
Target disease	B-cell NHL; CLL	B-cell NHL; CLL	B-cell NHL; CLL	CLL; T-cell NHL	AML
Usual regimen	• Monotherapy: 375 mg/m^2 weekly ×4 weeks • Combined with CT: 375 mg/m^2 on 1st day of each cycle • Maintenance: 375 mg/m^2 every 2–3 months	• Platelet count ≥150 × 10^9/l: 0.4 mCi/Kg • Platelet count 100–150 × 10^9/l: 0.3 mCi/Kg (Up to 32 mCi)	Needs dosimetry to establish the dose	30 mg 3 times per week ×8–12 weeks (starting with escalated doses: 3, 10 and 30 mg)	• Monotherapy: 9 mg/m^2 × two doses • Combined with CT: 3 mg/m^2 day 1
Main adverse effects	• Infusion toxicity (cytokine release syndrome)	Myelosuppression	Myelosuppression	• Infusion toxicity • Immunosuppression (opportunistic infections)	• Myelosuppression • Venoclusive disease
Cautions	Leukaemic expression	Bone marrow (+)	Bone marrow (+)	Opportunistic infections	Caution with thioguanine when used in combination

NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukaemia; AML, acute myeloid leukaemia; CT, chemotherapy.

[37] or MCP (mitoxantrone, chlorambucil and prednisolone) [35]}. Recent reports have pointed out that patients treated with rituximab containing regimens had better overall survival than those treated without rituximab [33, 34, 56–58]. In Table 3, main clinical trials in first-line are detailed. Based on these results, the combination of rituximab plus chemotherapy is considered the standard treatment in patients with FL; however, which chemotherapy regimen is the optimal to combine with rituximab remains controversial. In recent years, data from different institutions suggest a survival improvement in patients with FL, most likely due to the introduction of rituximab.

A new role for rituximab in patients with FL is as maintenance treatment after achieving a response with any rituximab-containing regimen [59–61]. There are data indicating advantages in terms of survival for patients receiving this maintenance therapy [31]. A multi-centre trial is ongoing to establish the role of maintenance with rituximab in first-line (PRIMA trial).

diffuse large B-cell lymphoma (DLBCL). After the results published by the French group GELA [39, 40], the new gold-standard in DLBCL is the combination of rituximab plus chemotherapy (CHOP being the most popular regimen). In a series of patients between 60 and 80 years of age diagnosed with DLBCL, R-CHOP demonstrated superiority over CHOP in terms of CR rate, primary resistance, event-free, progression-free, disease-free and overall survival (Table 3). This survey was confirmed in a similar group of patients in an American study [42]. Although the design was different (patients in response were randomized to no further treatment or maintenance with rituximab), those patients receiving rituximab either with CHOP or as maintenance had better survival. Moreover, the superiority of R-CHOP was shown in the setting of younger patients with low-risk DLBCL [41]. More recently, the addition of rituximab to a high-density regimen (CHOP-14) has demonstrated an improvement of response, disease-free and overall survival [44].

There are several ongoing studies on the role of rituximab in combination with other chemotherapy regimens, with autologous stem-cell transplantation, as well as the combination of different MoAbs plus rituximab-containing chemotherapy.

chronic lymphocytic leukaemia (CLL). In patients with CLL, rituximab alone has a discreet effect due, in part, to the low density of CD20 antigen in the surface of CLL tumour cells [13]. Higher doses (500–2250 mg/m^2) are necessary to increase the responses [51]. The combination with chemotherapy notably improves the effect of the latter. The combination of rituximab with fludarabine and cyclophosphamide shows a CR rate of about 70%, with a high proportion of patients reaching a molecular CR [52–54]. Certainly, the toxicity of the combination, especially in terms of immunosuppression, should be taken into account. The use of rituximab in maintenance therapy is currently under investigation.

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Table 3. Overall survival in selected randomized trials in first-line treatment comparing chemotherapy (CT) alone versus CT plus rituximab (R) in patients with different types of lymphoma

Disease	Reference	Regimen	n	Overall survival (CT) (%)	Overall survival (CT + R) (%)	Time of overall survival assessment	P
DLBCL	Coiffier 2002 [39] Feugier 2006 [40]	CHOP	399	45	58	5 years	0.007
	Habermann 2006 [42]	CHOP	632	58	67	3 years	0.05
	Pfreundschuh 2006 [41]	CHOP	824	84	93	3 years	<0.001
	Pfreundschuh 2006 [54]	CHOP-14	1222	67	75	3 years	0.003
MCL	Lenz 2005 [55]	CHOP	128	76	76	2 years	NS
	Herold 2004 [35]	MCP					
FL	Marcus 2005 [33] Marcus 2006 [56]	CVP	322	77	83	4 years	0.03
	Hiddemann 2005 [34]	CHOP	557	90	95	2 years	0.016
	Herold 2007 [57]	MCP	201	74	87	4 years	<0.001
	Buske 2006 [58]	CHOP	221	81	90	4 years	0.04

DLBCL, diffuse large B-cell lymphoma; MCL, mantle-cell lymphoma; FL, follicular lymphoma.

[^{90}Y]ibritumomab tiuxetan

[^{90}Y]Ibritumomab tiuxetan (Zevalin®) is a conjugate of murine anti-CD20 MoAb and the radionuclide ^{90}Y that delivers β-radiation in a 1–5 mm radium sphere. The infusion of the radiolabeled MoAb is preceded by two doses of a 'cold' unlabeled anti-CD20 (rituximab). Dosimetry estimation, mandatory for other radioisotopes, seems not to be necessary for [^{90}Y]ibritumomab tiuxetan [10]. The main toxicity is myelosuppression, especially when bone marrow is involved by the lymphoma. For this reason, patients with platelet count $<100 \times 10^9$/l and/or bone marrow involvement >25% should not receive [^{90}Y]ibritumomab tiuxetan. Nadir of neutrophils, haemoglobin and platelets occurs at 5–8 weeks after the dose. It is of note that no strong evidence of higher risk of myelodysplasia or acute leukaemia has been reported and the cases seen were most likely related to previous therapy with alkylating agents or fludarabine-containing regimens.

[^{90}Y]Ibritumomab tiuxetan has shown activity in relapsed FL patients, in whom CR rate and failure-free survival after this drug is longer than after rituximab [62, 63]. Noteworthy is that a proportion of relapsed patients had prolonged CR periods. At the present time, the established indication of this molecule is for relapsed or resistant to rituximab FL. In DLBCL and MCL [^{90}Y]ibritumomab tiuxetan shows some activity. The possible role for [^{90}Y]ibritumomab tiuxetan in consolidation of first response after conventional chemotherapy is currently being studied. In addition, the combination of [^{90}Y]ibritumomab tiuxetan with chemotherapy, including in the setting of autologous stem-cell transplantation, is also under investigation.

[^{131}I]tositumomab

[^{131}I]Tositumomab (Bexxar®) combines a murine anti-CD20 MoAb with ^{131}I. The dose depends on the dosimetry that is mandatory for this drug. Myelosuppression is the main limitation toxicity of [^{131}I]tositumomab [10].

In groups of patients with indolent lymphoma considered refractory to rituximab or with transformed lymphoma, [^{131}I]tositumomab induced overall responses rates of 65%, including 20% CR, with median response duration of 6–7 months. In untreated patients, single agent [^{131}I]tositumomab reaches a CR rate of 75%, with a median of progression-free survival of 6.1 years. On the other hand, [^{131}I]tositumomab has been combined with CHOP as consolidation, with 90% of overall response and 67% CR [64–68]. Currently there is an ongoing study that compares rituximab and [^{131}I]tositumomab combined with CHOP in FL patients not previously treated.

alemtuzumab

Alemtuzumab is a humanized IgG1 monoclonal antibody with specificity for the CD52 antigen, a glycosylphosphatidylinositol-anchored (lymphocyte-surface glycoprotein) which is widely expressed at high density on all human lymphoid cells (except plasma cells), as well as in eosinophils, monocytes, dendritic cells and macrophages.

Alemtuzumab is classically administered in a 2-hour intravenous infusion. Standard dose is 3 mg (1st day), 10 mg (2nd day) and 30 mg (3rd day); if it is well tolerated, 30 mg will be administered 3 days per week for 8–12 weeks. Subcutaneous administration is being increasingly used because the infusion syndrome is less frequent and does not need escalation of the dose. Pre-medication is usually given to prevent side effects of infusion (headache, mouth sores, rash, low blood pressure and fatigue), including dexchlorpheniramine, acetaminophen and hydrocortisone. Immunosuppression is the most important toxic effect, but it is manageable. Prophylaxis against *Pneumocystis carinii* should be used and prophylaxis against herpes zoster/simplex and fungal infections should also be considered. Weekly monitoring with cytomegalovirus polymerase chain reaction testing is recommended [10].

At present, alemtuzumab is licensed for patients with CLL, previously treated with alkylating agents and refractory to fludarabine. In this setting, reports shows that up to 87% of previously untreated patients respond to alemtuzumab with a 17% CR. Combination with other drugs, including fludarabine and rituximab, is the subject of in-progress investigations [69–72]. Strong immunosuppression is the main concern. Some responses have also been attained in refractory T-cell prolymphocytic leukaemia. Moreover, peripheral T-cell lymphomas constitute a group of poor-risk

patients in whom alemtuzumab alone or in combination may have an important role [69, 70]. Lastly, in the setting of allogeneic trasnplantation, alemtuzumab is used in order to do the T-cell depletion.

denileukin diftitox

Denileukin diftitox is a fusion protein that targets the diphtheria toxin to cells expressing the interleukin-2 receptor (CD25). When internalized into the cell, the drug inhibits protein synthesis. This MoAb has been used in cutaneous T-cell lymphoma, refractory B and T-cell lymphomas and in fludarabine-resistant CLL [73].

other antibodies

Due to the content limitation required for this review it would be impossible to mention many other MoAbs that are being used in clinical trials. However, among these it would be of interest to mention the following molecules: the new different anti-CD20 MoAb (including the fully humanized IMMU-106 hA20), anti-CD22 (unconjugated epratuzumab and calicheamicin conjugated CMC-544), anti-CD30 (SGN-30 and iratumumab), anti-CD40 (SGN-40), anti-CD80 (galiximab), anti-CD2 (siplizumab) and anti-CD4 (L3T4). Bevacizumab, a VEGF (vascular endothelial growth factor) inhibitor may play also an interesting anti-lymphoma role in the future.

acute leukaemias

Table 1 shows a list of potential targets to block by MoAb in acute leukaemias. Gemtuzumab ozogamicin is nowadays the only one being used in the clinical practice out of clinical trials.

gemtuzumab ozogamicin

Gemtuzumab ozogamicin is an immunoconjugate of an anti-CD33 antibody chemically linked to a potent cytotoxic agent, calicheamicin. It appears to be particularly active in patients with acute leukaemia. CD33 antigen is expressed on the surface of leukaemic cell blasts in more than 90% of patients with acute myeloblastic leukaemia [7]. It is administered as a 2-hour intravenous infusion. The dose infused is 6–9 mg/m^2 in two administrations. Side effects include myelosuppression, headache, rash, low blood pressure, increased levels of hepatic enzymes and fatigue. A unique potential toxicity is a veno-occlusive-like disease that may be very severe and always problematic in patients who subsequently undergo through haematopoietic stem cell transplantation.

As single agent treatment (9 mg/m^2 in two doses), 26% of patients receiving gemtuzumab ozogamicin reached CR that lasted for a median of 6 months [74]. The feasibility of combining gemtuzumab ozogamicin (at a dose of 3 mg/m^2) with chemotherapy has been recently demonstrated with a reasonable toxicity [75]. In this sense, regimens containing thioguanine should be avoided because of grades 3–4 liver toxicity.

conclusion

Much progress has been made during the last few decades in the treatment of haematological malignancies. MoAbs represent a major advance towards a targeted therapy that can dramatically improve the anti-tumour effect with a substantial reduction of toxicity derived from therapy. In general, MoAbs are safe, well-tolerated, and have activity in a variety of clinical settings. The combination with chemotherapy as well as the combination of different MoAbs is not a dream for the future, but a solid reality for many patients with lymphoma or leukaemia.

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